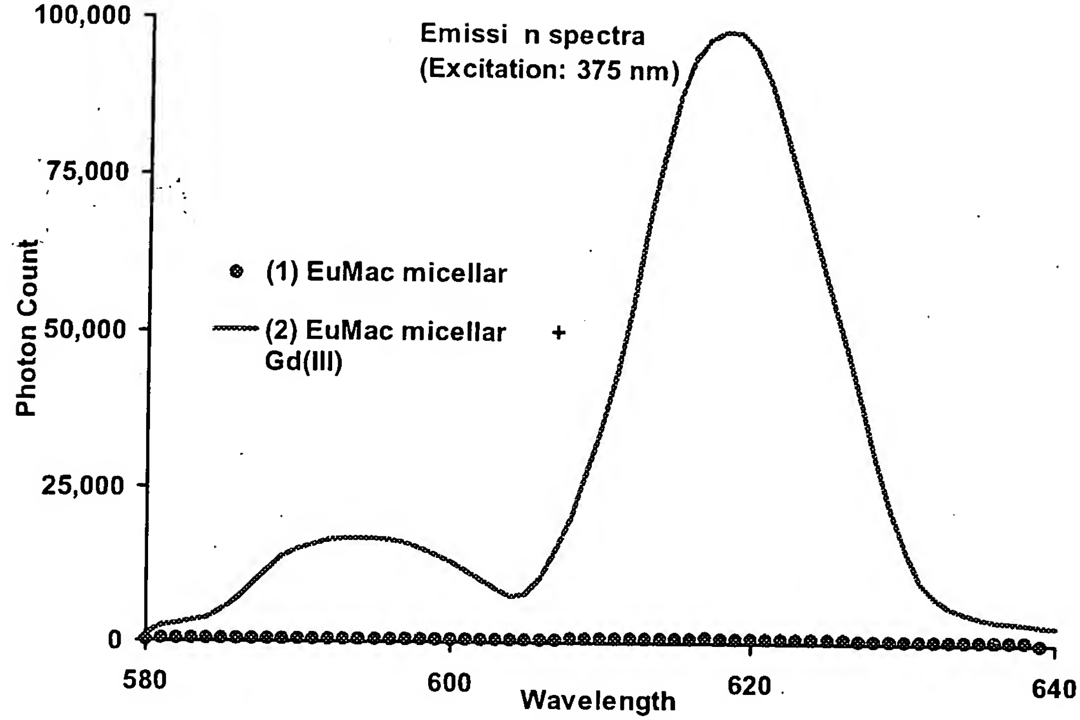

Figure 1: Emission spectra (Excitation: 375 nm) of: (1) A solution of [Eu-macrocycle(acetate)$_2$](acetate) (2.3×10^{-7} M) in the optimized-cofluorescence matrix without Gd(III). (2) An identical solution but with Gd(III) chloride (1.2×10^{-4} M). In (2), the integrated emission intensity between 613 and 623 nm is increased over 100-fold by the addition of Gd(III).

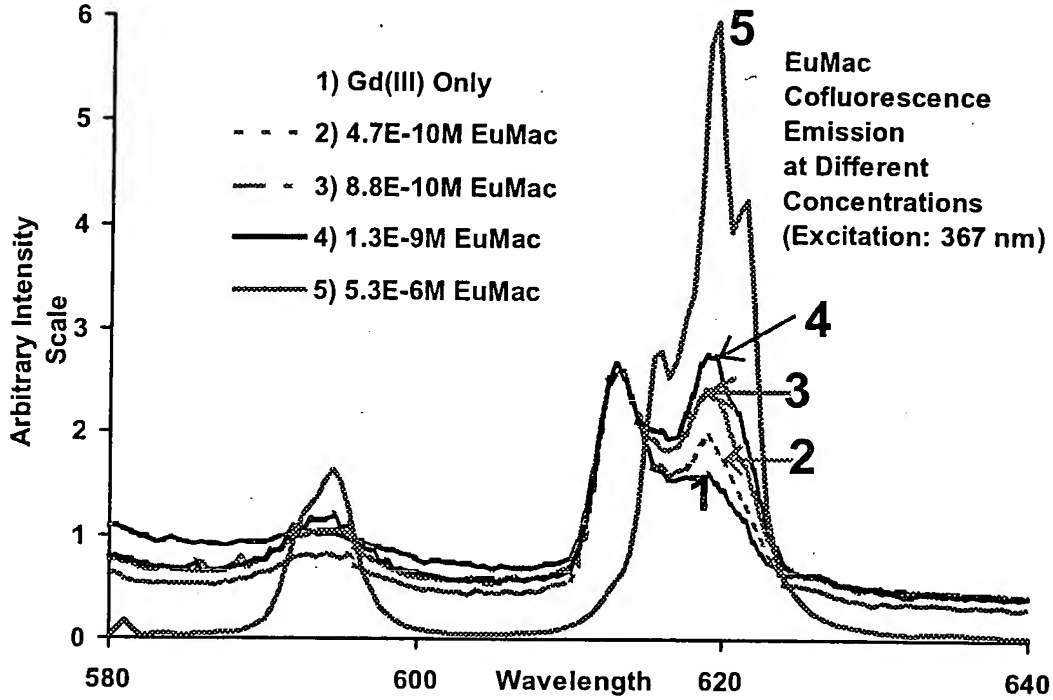

Figure 2: Emission spectra (excitation, 375 nm) of cofluorescence-optimized solutions containing "free" Eu(III) (approximately 4 x 10^{-11}M) as contaminant in the Gd(III) and the [Eu-macrocycle(acetate)$_2$](acetate) at four different concentrations, 4.7 x 10^{-10} M, 8.8 x 10^{-10} M, 1.3 x 10^{-9} M, and 5.3 x 10^{-6} M. The peak maximum for the $^5D_0 \rightarrow {}^7F_2$ transition is 614 nm for the Eu(III) contaminant and 619 nm for the Eu-macrocycle. Because they had the same Eu(III) contaminant, spectra 1 to 4 were normalized to the same peak height at 614 nm. Spectrum 5 (the highest concentration of the Eu-macrocycle) was scaled to permit comparison of the spectra.

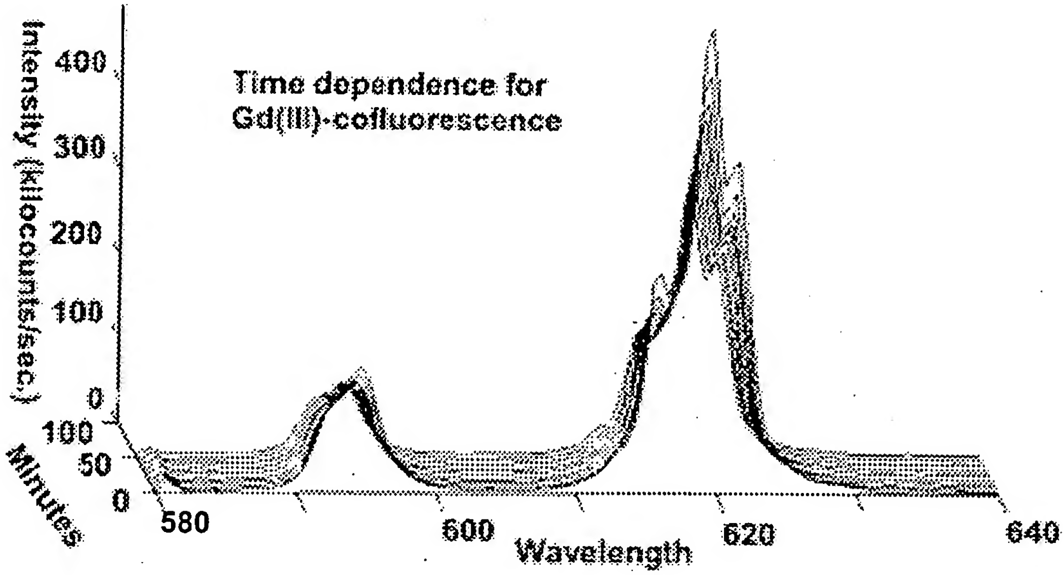

Figure 3. Time-dependence plot for the emission intensity of the [Eu-macrocycle(acetate)$_2$](acetate) complex in a Gd-containing optimized cofluorescence solution. Only one band arising from the $^5D_0 \rightarrow {}^7F_0$ transition of the Eu-macrocycle transition occurs at ca. 580 nm, showing that only one emitting species is present. Furthermore. the peak pattern of the band corresponding to the $^5D_0 \rightarrow {}^7F_2$ transition is constant in time, even though the intensity decreases, showing that the chemical nature of the emitting species remains unchanged.

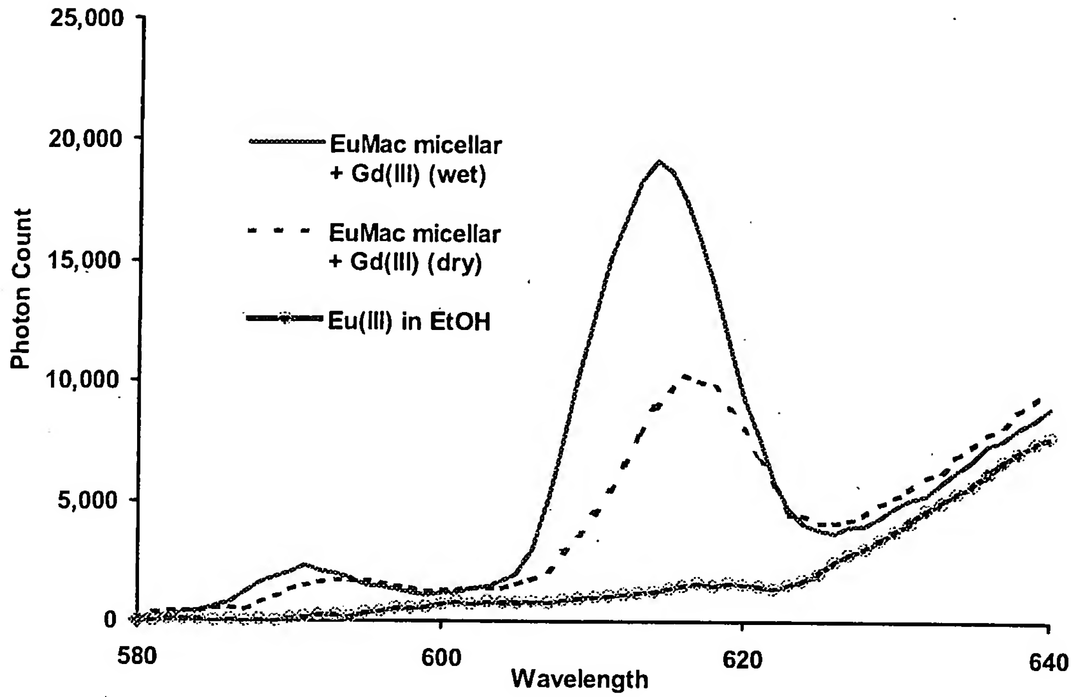

Figure 4. Reflectance emission spectra (Excitation: 375 nm) of wet and dry spots obtained from a cofluorescence-optimized aqueous micellar solution of [Eu-macrocycle(acetate)$_2$](acetate) (2.3×10^{-7} M), and of a wet spot from an ethanol solution of Eu(III) (2.3×10^{-7} M) with only HTTFA added. Spectra were recorded under identical instrumental settings and the background from the paper was subtracted; however, the reflectance behavior of the paper changes upon drying. The rise in the curve above 630 nm is due to scattering from the paper.

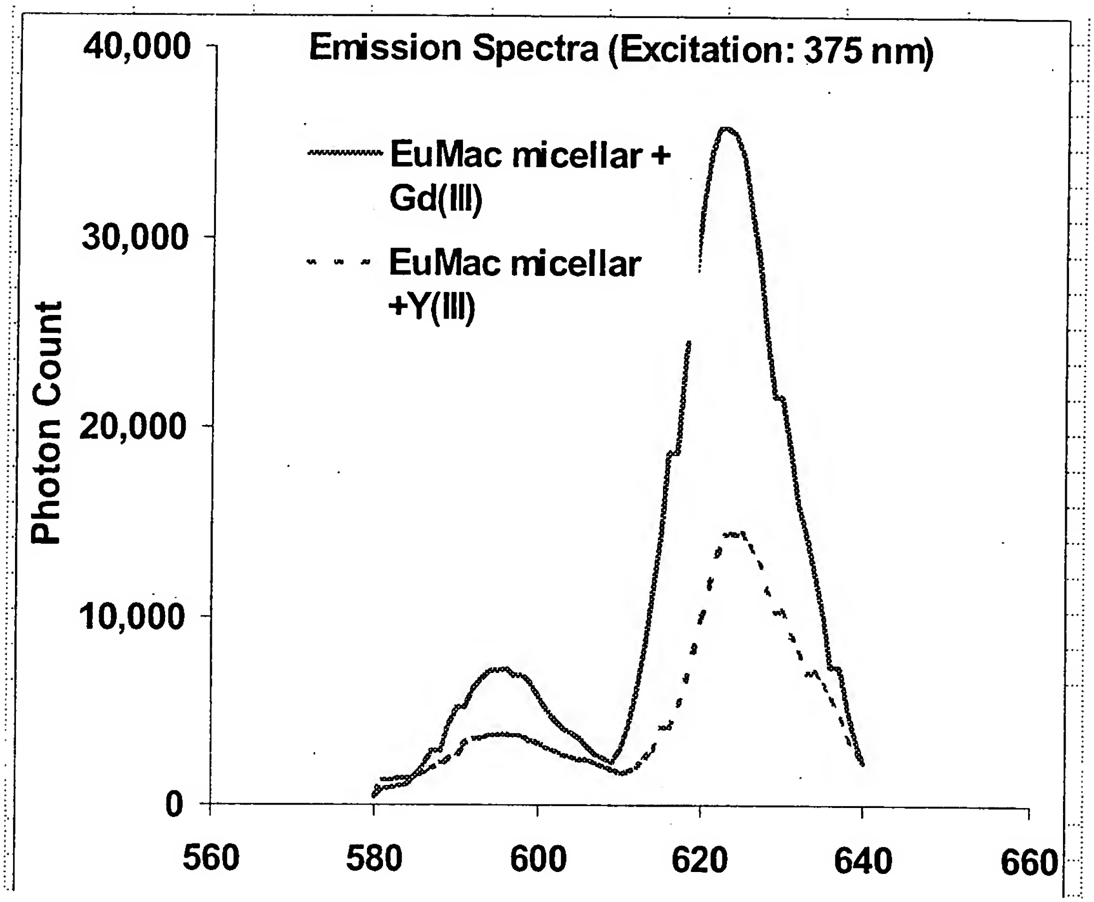

Figure 5. Emission spectra (Excitation: 375 nm) of [Eu-macrocycle(acetate)$_2$](acetate) in two cofluorescence solutions, one containing Gd(III) and the other containing Y(III) as the energy transfer donor. (All other reagents are present at the same concentrations, see Table 1). The Gd(III) provides significantly stronger, 2.6 fold, enhancement relative to Y(III).

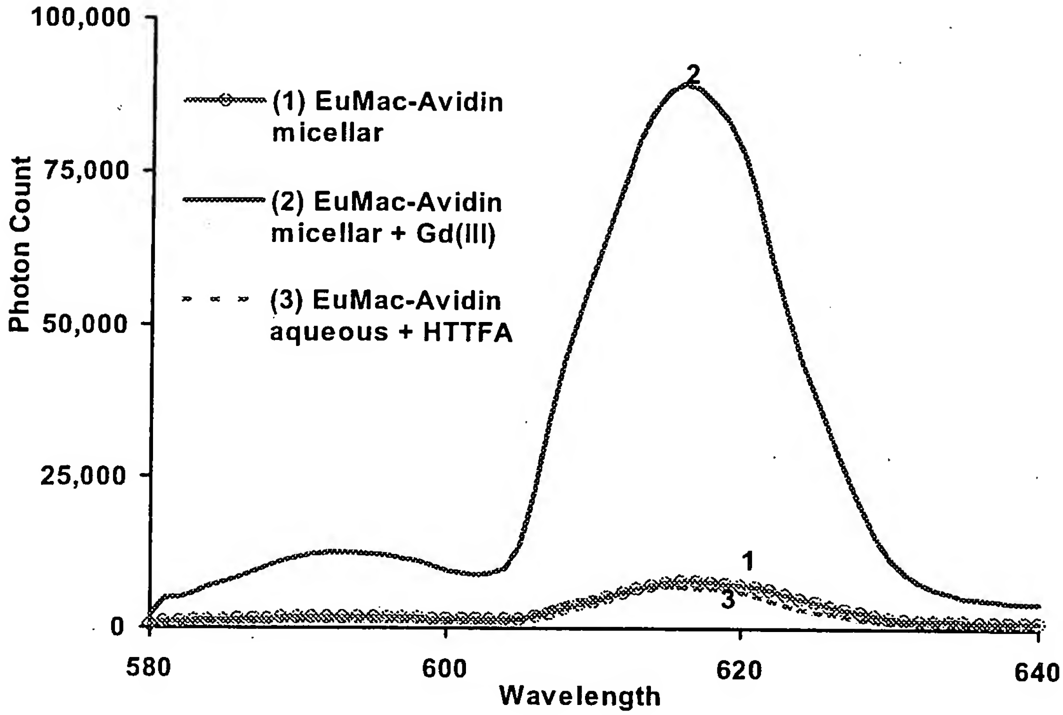

Figure 6. Emission spectra (Excitation: 365 nm) of the EuMac-Avidin at a concentration of 2.2×10^{-6} mol europium)/L in: (1) A cofluorescence-optimized aqueous micellar solution. (2) Identical to the preceding solution but with Gd(III) chloride (1.2×10^{-4} M). (3) An aqueous buffered solution with only HTTFA added.

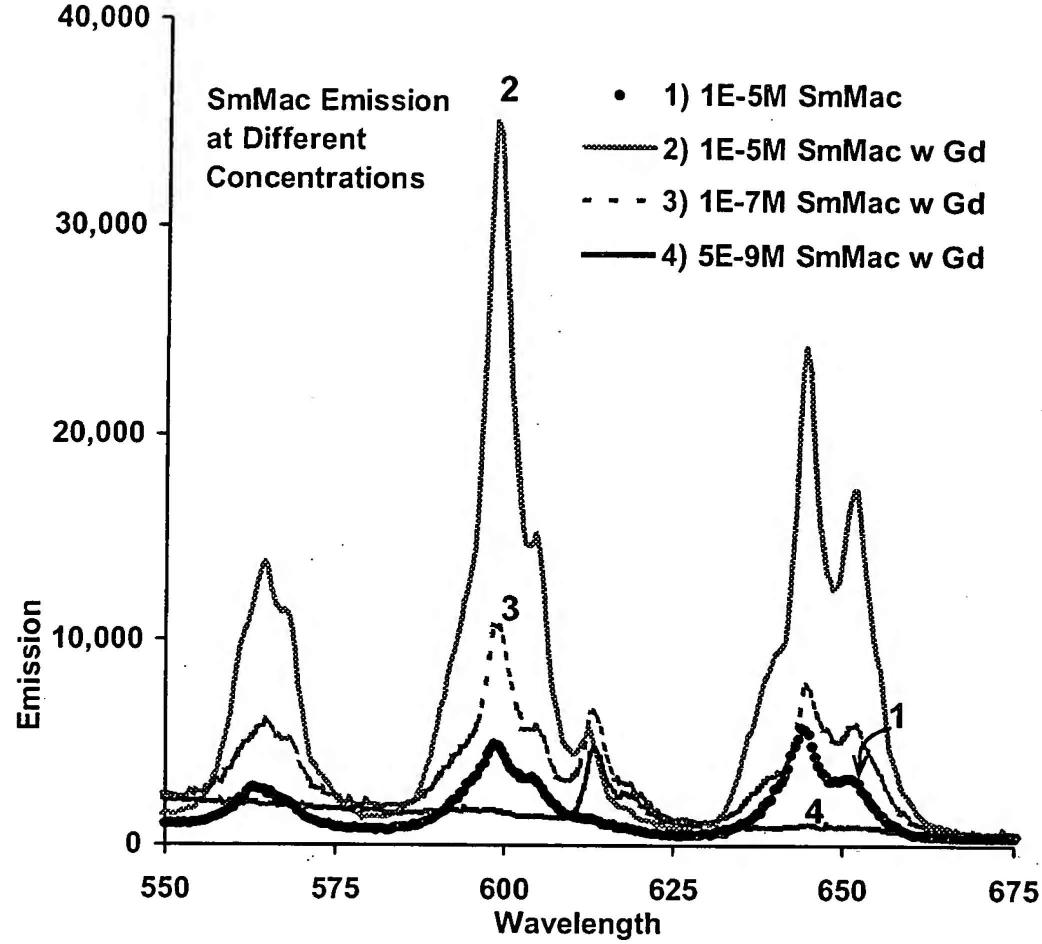

Figure 7. Emission spectra (excitation, 367 nm) of [Sm-macrocycle(acetate)$_2$](acetate) at three different concentrations, 1×10^{-5} M, 1×10^{-7} M, 1×10^{-9} M, in cofluorescence-optimized solutions with and without Gd(III). The solutions that contain gadolinium also contain "free" Eu(III) (approximately 4×10^{-11}M) as contaminant. The SmMac spectrum shows three emissions at {563},{599}, and {644} nm, arising from the $^4G_{5/2} \rightarrow {}^6H_{5/2}$, $^4G_{5/2} \rightarrow {}^6H_{7/2}$, and $^4G_{5/2} \rightarrow {}^6H_{9/2}$ transitions of Sm(III); the constant-intensity emission at 614 nm arises from the $^5D_0 \rightarrow {}^7F_2$ transition of the Eu(III) contaminant.

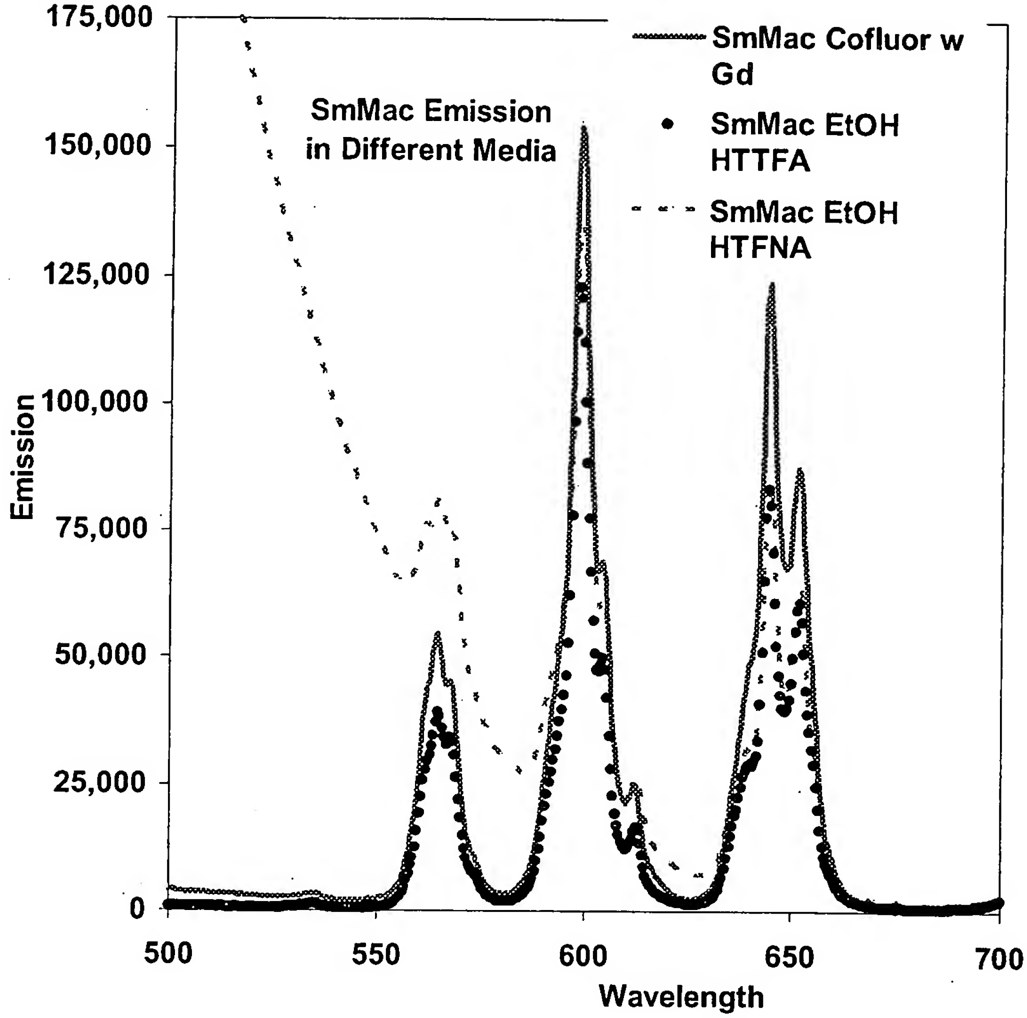

Figure 8. Emission spectra (excitation, 367 nm) of [Sm-macrocycle(acetate)$_2$](acetate) ($1.0x10^{-4}$ M) in: (a) a Gd-containing optimized cofluorescence solution, (b) an ethanol solution containing HTTFA ($4.0x10^{-4}$ M), and (c) an ethanol solution containing HTFNA ($4.0x10^{-4}$ M).

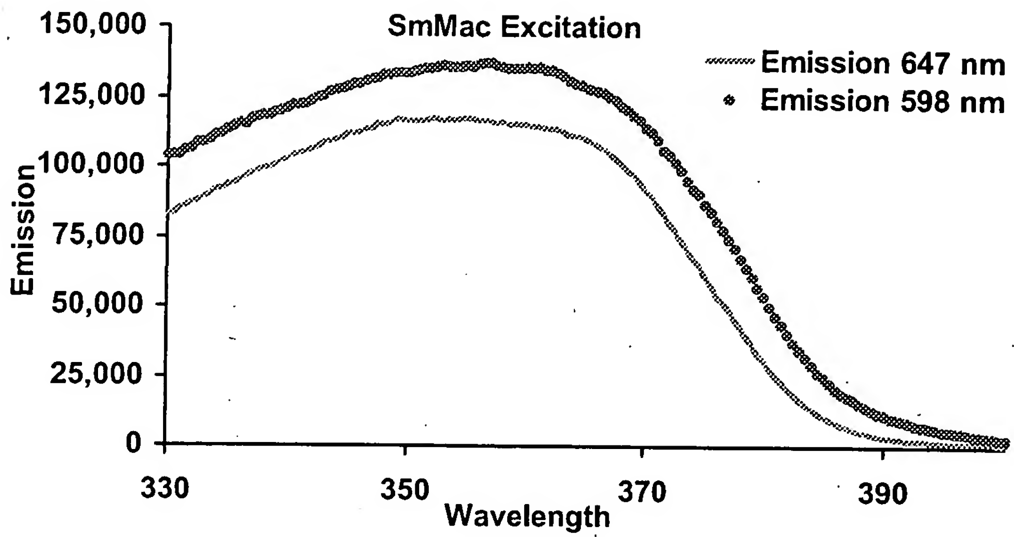

Figure 9. Excitation spectra of [Sm-macrocycle(acetate)$_2$](acetate) (1.0×10^{-4} M) in an ethanol solution containing HTTFA (4.0×10^{-4} M), for emission of 598.5 and 647.0 nm, respectively. The shapes of the excitation spectra including their maxima for the two emissions are identical.

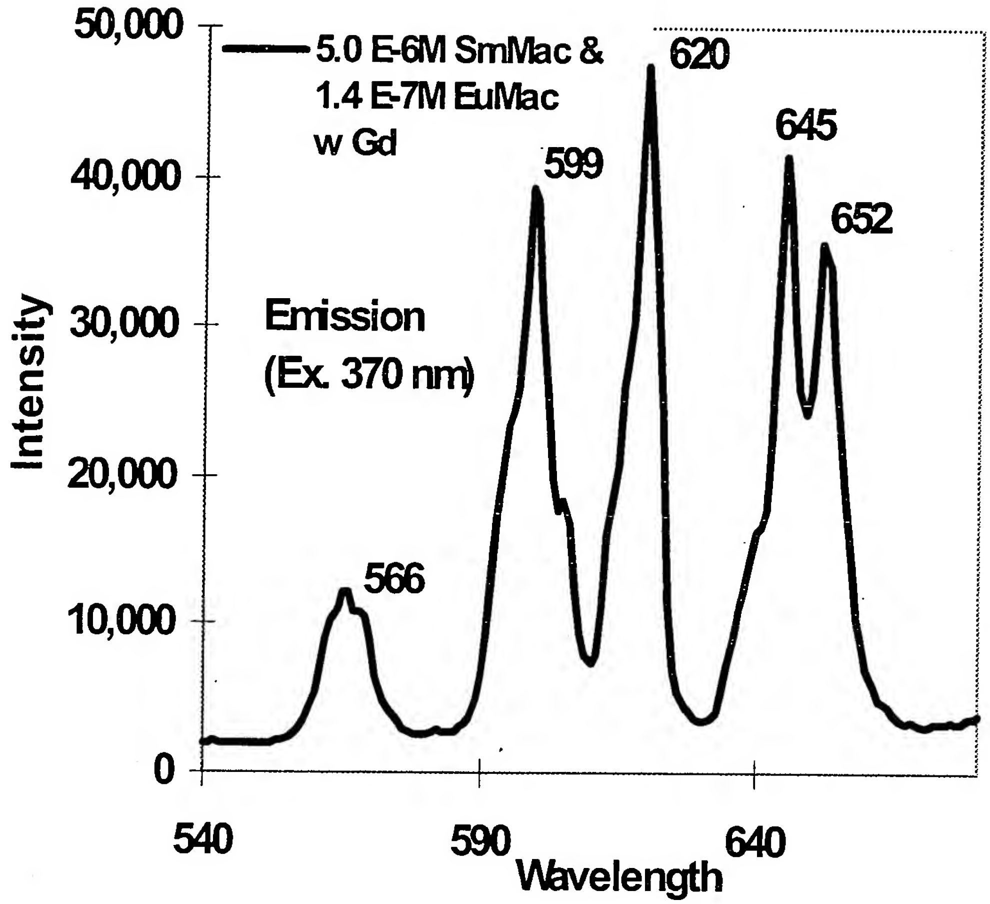

Figure 10. Emission spectrum (excitation, 370 nm) of a gadolinium-induced cofluorescence solution containing 5.0×10^{-6} M [Sm-macrocycle(acetate)$_2$](acetate) and 1.4×10^{-7} M [Eu-macrocycle(acetate)$_2$](acetate); all other components as in Table 1. The SmMac and EuMac complexes were combined prior to micelle formation and their concentrations were chosen to provide approximately equal emission intensities in the mixture. The $^5D_0 \rightarrow {}^7F_2$ (619 nm) emission of the EuMac species is well separated from the neighboring $^4G_{5/2} \rightarrow {}^6H_{7/2}$ (599 nm), and $^4G_{5/2} \rightarrow {}^6H_{9/2}$ (644 and 652 nm) emissions of the SmMac, so that the intensities of each emission can be measured independently. The excitation and emission slits were respectively 16 and 2 nm.

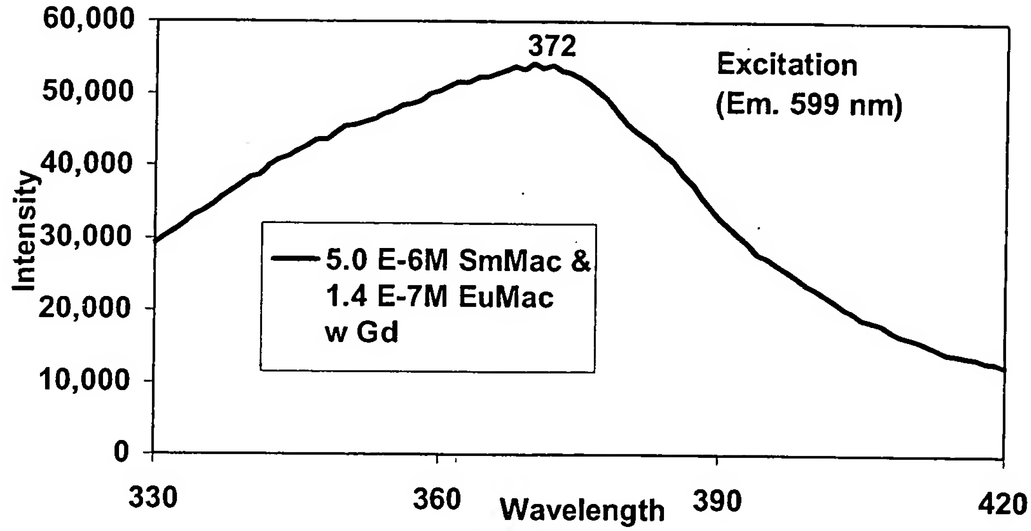

Figure 11. Excitation spectrum of the SmMac complex (emission, 599 nm) in a gadolinium-induced cofluorescence solution containing $5.0x10^{-6}$ M [Sm-macrocycle(acetate)$_2$](acetate) and $1.4x10^{-7}$ M [Eu-macrocycle(acetate)$_2$](acetate). All other components had the concentrations given in Table 1 and the SmMac and EuMac were combined prior to micelle formation. The excitation and emission slits were 8 and 4 nm, respectively. The excitation spectrum of the EuMac complex is nearly identical.

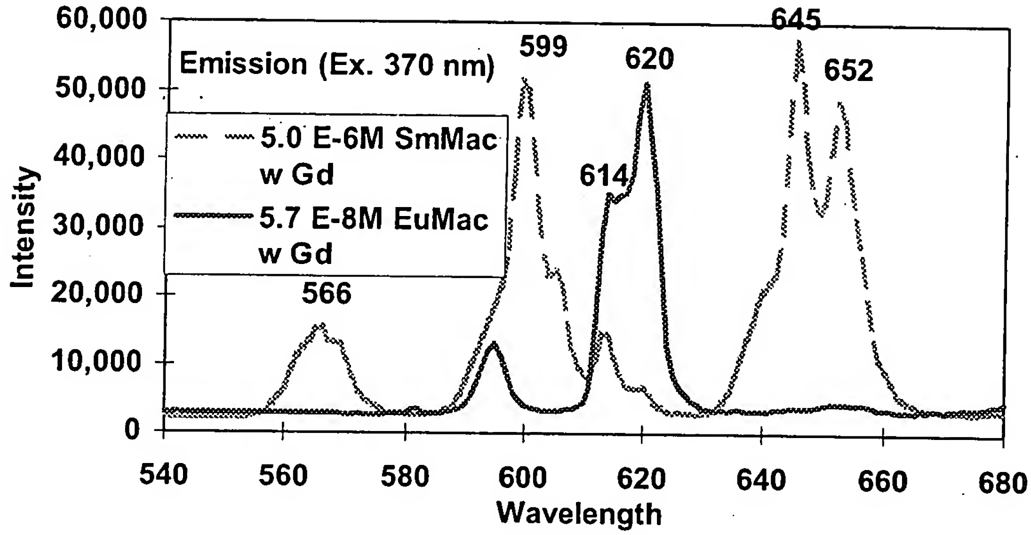

Figure 12. Composite of the emission spectra (excitation, 370 nm) of two gadolinium-induced cofluorescence solutions, one containing SmMac (5.0×10^{-6} M) alone and the other containing EuMac (5.7×10^{-8} M) alone. The concentrations of the macrocyclic complexes were chosen to provide approximately equal emission intensities.

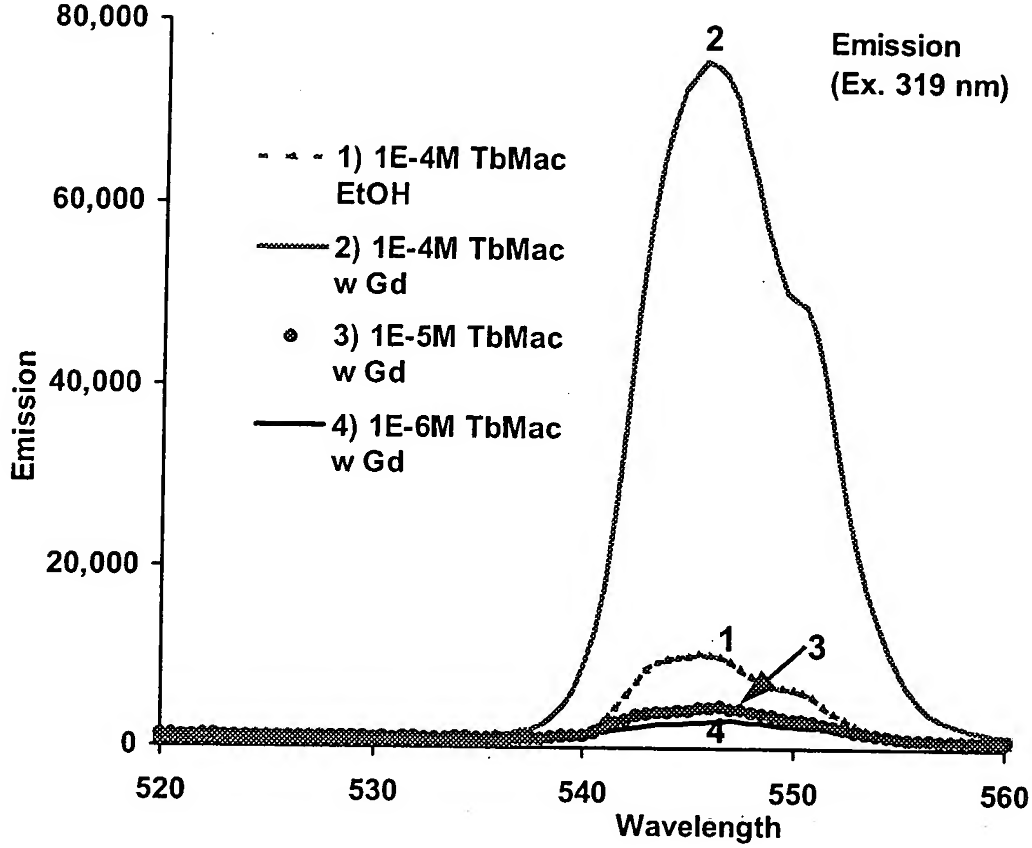

Figure 13. Emission spectra (excitation, 319 nm) of: (1) an ethanol solution of TbMac, 1.0×10^{-4} M with only HPTFA (8×10^{-4} M), (2) (3) and (4) Gd-containing cofluorescence-optimized solutions of TbMac, 1.0×10^{-4} M, 1.0×10^{-5} M, 1.0×10^{-6} M, respectively.